

Expert Opinion

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Somatostatin receptor agonists and antagonists

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Somatostatin is a cyclic peptide that is widely distributed in the CNS, the periphery and in a variety of tumours. Two biologically active forms, somatotropin release-inhibiting factor (SRIF)-14 and SRIF-28, exert their effects through activation of five G-protein-coupled receptor subtypes (sst₁ – sst₅). These peptides act as neurotransmitters or hormones and inhibit the secretion of other peptides, such as insulin, growth hormone and glucagon. Metabolically stable peptide and structurally diverse non-peptide analogues have been developed as subtype-selective agonists and antagonists. The availability of these novel SRIF analogues will greatly facilitate our understanding of the function and role of specific SRIF receptors. SRIF analogues offer therapeutic potential in the regulation of hormone secretion, diseases of the CNS and periphery and in the treatment and diagnosis of various tumours. This review will focus on an overview of SRIF, new developments related to SRIF role and function and the discovery of novel peptide and non-peptide agonists and antagonists.

Keywords: somatostatin, somatostatin receptors (sst), somatotropin release-inhibiting factor (SRIF), SRIF agonists, SRIF antagonists

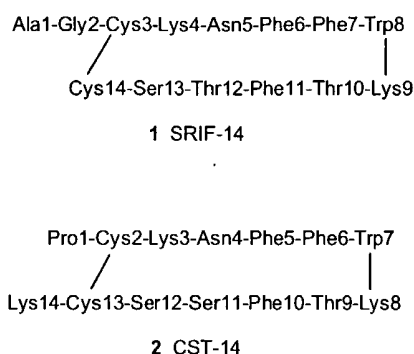
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1. Introduction

1.1 Endogenous peptides

Somatostatin (somatotropin release-inhibiting factor [SRIF]; compound 1) is a cyclic tetradecapeptide that was isolated from hypothalamic extracts by Brazeau *et al.* [1]. SRIF was shown to exist in the two biologically active forms SRIF-14 and SRIF-28, an extended form of 14 amino acids from the *N*-terminal end of SRIF-14. Both forms are derived from the polypeptide precursor presomatostatin and they contain one internal disulfide bond between residues 3 and 14. These peptides may function as neurotransmitters or hormones.

Cortistatin-14 (CST-14; compound 2) is a recently discovered neuropeptide from rat brain that has 11 of the 14 amino acids in common with SRIF-14 [2]. The name of this peptide is derived from its predominate cortical expression and its ability to depress cortical activity [3]. Subsequent studies by Fukusumi *et al.* [4] demonstrated that a human preprocortistatin (PPCST) leads to a 17 amino acid peptide, CST-17. Binding studies in CCL-39 (Chinese hamster lung fibroblast) cells demonstrated that both SRIF-14 and CST-14 have a high affinity for SRIF receptors. None of the SRIF receptor subtypes showed any selectivity for either SRIF-14 or CST-14 [5]. The post-translational processing of PPCST from rat brain was studied in AtT-20 cells. Analysis of the processed products revealed that CST-14, CST-29 (an extended form of 15 amino acids from the *N*-terminal end of CST-14) and unprocessed PPCST were produced in a ratio of (41:45:55). However, CST-14 was the predominately-released product under basal- and forskolin-induced experimental conditions [6]. Another recent study demonstrated that CST-14, but not SRIF, binds to growth hormone secretagogue receptors (GSHRs) from the human pituitary. These workers



speculated that CST-14 may play a modulatory role on GSHR activity in the human pituitary [7]. Dalm *et al.* [8] used reverse transcription-polymerase chain reaction (RT-PCR) techniques to investigate the expression of SRIF and CST in a variety of human lymphoid tissues and immune cells. SRIF was found only in the human thymus, whereas CST mRNA was found in the lymphoid tissues, immune cells and bone marrow. Furthermore, CST mRNA was shown to be upregulated during differentiation of monocytes into macrophages and dendritic cells. Using radioaudiography, CST displaced [¹²⁵I]-Tyr3 octreotide in human thymic tissues and on SRIF receptor-expressing cells. This study suggests that CST may be the endogenous ligand at SRIF subtype 2 receptors in the human immune system. However, the question remains unanswered as to whether specific CST receptors exist.

1.2 SRIF receptor subtypes

SRIF exerts its effects by binding to a family of widely distributed, structurally related SRIF receptors (sst). Five subtypes of receptors have been cloned and identified. The receptors belong to the G-protein-coupled superfamily and are designated sst₁ – sst₅ [9,10]. On the basis of structural and pharmacological aspects, sst₂, sst₃ and sst₅ comprise the SRIF₁ receptor family, while the sst₁ and sst₄ subtypes comprise the SRIF₂ receptor family [9,11]. These major families of SRIF receptors are distinguished on the basis of the increased affinity of the SRIF₁ family for hexapeptide and octapeptide analogues of SRIF [12]. Two isoforms of the sst₂ receptor that differ only in their intracellular C termini are found in the mouse and rat. These splice variants are designated sst_{2A} and sst_{2B}, respectively [13,14].

G-protein-linked second messenger systems, including adenylyl cyclase, Ca²⁺ and K⁺ ion channels, phospholipase C (PLC) and mitogen-activated protein kinase (MAPK), have been implicated in the signal transduction of SRIF receptors [15,16]. Agonist binding at ssts results in potent inhibition of adenylyl cyclase. SRIF receptor signalling also causes induction of several phosphatases, such as the serine–threonine phosphatases [17]. Other signalling pathways for ssts include phospholipase A₂-dependent stimulation of arachidonate production in hippocampal neurons and PLC-mediated

stimulation of inositol-1,4,5-triphosphate (IP₃) formation in astrocytes and smooth muscle cells [18,19].

Recent studies suggest that individual receptor subtypes can activate more than one G protein and G-protein-linked signalling cascade. The fact that single cells express more than one receptor subtype has presented perplexing questions such as whether multiple ssts in the same cell are redundant or whether they coordinate functionally for greater signalling diversity. SRIF receptor subtypes sst_{2A} and sst₃ were shown to exist as either homodimers or as heterodimers when expressed in human embryonic kidney (HEK)-293 cells. Although the sst_{2A}–sst₃ heterodimer exhibited properties of the sst_{2A} receptor subtype, the heterodimer was devoid of sst₃ receptor function [20]. A subsequent study showed that the sst_{2A} receptor forms a stable heterodimer with the μ -opioid receptor (MOR1). Unlike the situation with the sst_{2A}–sst₃ heterodimer, the sst_{2A}–MOR1 heterodimer retained ligand binding and coupling properties of both receptors. Interestingly, heterodimerisation promoted cross-modulation of phosphorylation, internalisation and desensitisation of these receptors [21]. These workers speculated that heterodimerisation might be a means of modulation of phosphorylation and desensitisation of G-protein-coupled receptors.

1.3 Distribution and physiological functions of somatostatin receptors

The five SRIF receptor subtypes are found in the CNS, periphery and in various tumours [17]. All five receptor subtypes are expressed in the brain. Areas rich in the expression of each individual subtypes genes are the cerebral cortex for subtypes 1 and 2, amygdala for subtypes 1 and 3 and the hypothalamus and preoptic area for subtype 5 [22,23]. Compared with the other subtypes, the expression of subtype 4 in the brain is relatively poor [24]. The sst₂ and sst₅ receptors are the major subtypes found in human pituitary cells, with a predominant expression of sst₅ [25]. The ssts are present in the endocrine and exocrine pancreas and on growth plates of long bones [26]. The human stomach expresses all five subtypes, whereas only subtypes 1 – 4 have been identified in the rat stomach [27]. The rat small intestine displays moderate levels of sst₁ and sst₅, low levels of sst₃ and sst₄, but no expression of sst₂ [17,28]. The adrenal glands display high levels of sst₂ and moderate levels of sst₁ and sst₃. In addition, ssts are found in the liver and spleen (sst₃) and in the lung and heart (sst₄) [16]. Recent studies have shown the presence of ssts in human vascular tissue. Human blood expresses high levels of sst₁ with much less expression of sst₂ and sst₄. Subtypes 3 and 5 were not detected in either normal or diseased blood vessels [29]. SRIF receptor subtypes 3, 4 and 5 are found in mouse proximal tubules, while subtypes 1 and 2 have been found in rat or human kidneys, primarily in the collecting ducts [30].

Specific physiological functions have only been attributed to SRIF receptor subtypes 2 and 5. The release of growth hormone (GH) from primary cultures of anterior pituitary was

shown to involve both ss_{t2} and ss_{t5} [31]. Culler *et al.* [32] showed that SRIF subtypes 2 and 5 are responsible for suppression of GH release in the human pituitary. Using primary cultures of cells from acromegalic patients, these workers showed that individuals who failed to respond fully to SRIF analogue therapy had dramatically reduced ss_{t2} receptor expression. Strowski *et al.* [33] used SRIF receptor 2 knockout (SSTR2 KO) mice to show that glucagon release in mouse islets was primarily mediated by ss_{t2} , whereas insulin secretion was regulated by ss_{t5} . A subsequent study [34] using SRIF receptor 5 knockout (SSTR5 KO) mice, demonstrated that ss_{t5} mediates SRIF inhibition of pancreatic insulin secretion and plays an important role in the regulation of glucose homeostasis and insulin sensitivity. Subtype-selective analogues were used to identify the ssts involved in regulation of adrenocorticotrophic hormone (ACTH) release from AtT-20 cells, a model for cell line pituitary corticotropes. SRIF and non-peptidyl ss_{t2} agonists were shown to inhibit forskolin- and corticotropin-releasing hormone (CRH)-induced increases in intracellular cAMP. Also, non-peptide ss_{t2} - and ss_{t5} -selective agonists potently inhibited CRH-induced ACTH release using this model [35].

Glucagon-like peptide (GLP)-1 is a postprandial proglucagon-derived peptide that is released from the endocrine cells of the gastrointestinal tract. This peptide acts to lower blood glucose levels by stimulation of insulin release from pancreatic islet β -cells [36]. In a rat insulinoma cell line R1Nm5F model, GLP-1 stimulated insulin release, cell proliferation and increased intracellular cAMP concentrations. The R1Nm5F cell line primarily contains ss_{t1} and ss_{t2} receptors. Administration of selective ss_{t1} and ss_{t2} , but not ss_{t3} , selective non-peptidyl agonists or a peptide SRIF analogue returned GLP-1-stimulated insulin release, cell proliferation and intracellular cAMP to baseline levels [37]. Using a fetal rat intestinal culture, Chisholm and Greenberg [38] demonstrated that SRIF-28 dose-dependently inhibited GLP-1 secretion which was stimulated by gastrin-releasing peptide. This inhibition was more potent with SRIF-28 than with SRIF-14. Conversely, GLP-1 increased SRIF-14 and SRIF-28 by 5- and 3-fold, respectively. The ss_{t5} -selective peptide analogue BIM-23268 was almost as effective as SRIF-28 at inhibiting GLP-1 secretion. The results of this study suggest that GLP-1 inhibition is regulated by the action of SRIF-28 on ss_{t5} receptors. The authors concluded that GLP-1 secretion is autoregulated by ss_{t5} activation and that a feedback loop exists between GLP-1 and SRIF-28 that is under ss_{t5} control.

Ghrelin, a 28 amino acid peptide, is a natural ligand at the GHSR that strongly stimulates the secretion of GH in both animals and humans. In a recent study in human volunteers, SRIF-14 was shown to almost completely suppress GH-releasing hormone (GHRH)-induced GH release [39]. A subsequent study by these workers evaluated the effects of intravenous administration of SRIF-14 or CST-14 on ghrelin, GH and insulin and glucose levels in young men. Both CST-14 and SRIF-14 inhibited circulating levels of ghrelin by ~ 55%. The

ghrelin levels remained lower than baseline values even after the 30-min infusion of SRIF-14 or CST-14 was terminated [40].

SRIF analogues were found to bind with high affinity to rabbit retinal membranes. Furthermore, SRIF-14 and subtype-2-selective agonists were shown to stimulate GTPase activity in a concentration-dependent manner. The results of this study suggested that SRIF produces its effects in the rabbit retina by binding to ss_{t2} receptors that are functionally coupled to a G_{α} protein [41]. In a study using retinæ from SSTR2 KO mice, SRIF-14 and its analogues failed to alter glutamate release. However, in wild-type (WT) mice, SRIF-14 and its analogues displayed high selectivity for ss_{t2} in mouse retinæ and also inhibited potassium-evoked release of glutamate [42]. Studies using the rat retina showed that SRIF-14 and an ss_{t2} -selective non-peptide agonist stimulated nitric oxide production, whereas an ss_{t2} -selective antagonist blocked this effect. These studies provide strong evidence that SRIF-14 regulates nitric oxide synthesis in the rat retina through activation of subtype 2 receptors [43].

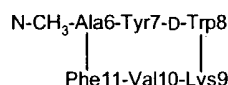
SRIF influences carcinogenesis, tumour growth and tumour metastasis through a combination of direct and indirect mechanisms [16]. This peptide inhibits cell secretion and prevents cell proliferation by inducing cell cycle arrest or apoptosis. Most human tumours express one or more SRIF receptors that are responsive to SRIF or its analogues. Although the direct effects are mediated through ssts, SRIF and its analogues may exert an indirect effect by inhibiting hormone and growth factor secretion, angiogenesis and immune cell activity in non-tumour cells [17]. Reubi *et al.* [44] used autoradiography with [125 I]-Tyr-[Leu8,D-Trp22,Tyr25] SRIF-28 and its displacement with receptor-selective ligands to study sst receptor protein expression in approximately 200 tumour types. This study showed that ss_{t2} was expressed in a majority of tumours. Teixeira *et al.* [45] showed that HL-60 cells contained ss_{t2} receptors and that treatment with an SRIF-14 analogue resulted in increased cell death through apoptosis. This effect was attributed to activation of subtype 2 receptors through a mechanism independent of p53. In a project to study the localisation of SRIF and ssts in benign and malignant ovarian tumours, Hall *et al.* [46] found that SRIF correlated well with the expression of ss_{t1} , ss_{t2} , ss_{t3} and ss_{t5} receptors in epithelial, vascular and stromal compartments of these tumours. Recently, Hansson *et al.* [47] reported, for the first time, the presence of ss_{t2} and ss_{t4} mRNA in benign prostatic hyperplasia (BPH) and malignant cells of prostate cancer tissue. This research showed that ss_{t2} and ss_{t4} receptors were upregulated in malignant cells, leading to speculation that subtype 2- and 4-selective agonists could have therapeutic potential in the treatment of prostate cancer.

2. Somatotropin release-inhibiting factor analogues with affinity for somatostatin receptors

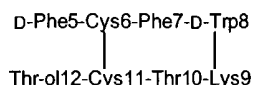
2.1 Peptide agonists

Poor bioavailability and rapid inactivation by peptidases severely limit the therapeutic effectiveness of SRIF. Thus, the

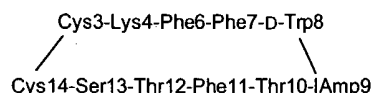
Somatostatin receptor agonists and antagonists



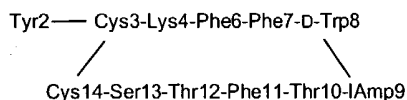
3 MK-678



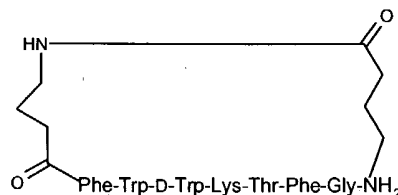
4 Octreotide



5 CH-275



6



7 PTR-3173

search for metabolically stable peptidomimetics has been the focus of extensive research [48]. Structure-activity relationship (SAR) studies have shown that the Trp8 and Lys9 residues are essential for biological activity and that the tetrapeptide Phe7-Trp8-Lys9-Thr10 comprises the critical β -turn of SRIF [49]. This information led to the development of the hexapeptide MK-678, compound 3 [50], and the cyclic octapeptide octreotide (SMS-201955; compound 4) [51], both potent SRIF agonists. The des-amino acid (1,2,5)[D-Trp8,Iamp9], CH-275 (compound 5) and its [^{125}I -Tyr2] analogue, compound 6, were shown to bind with high affinity and selectivity at sst_1 receptors in displacement studies using [^{125}I -Tyr11]-SRIF with dissociation constant (K_d) values of 1.8 ± 0.7 nM and 0.5 ± 0.1 nM, respectively [52]. Additional studies from these researchers demonstrated that des-amino acid1,2,5[D-Trp8,Iamp9],[^{125}I -Tyr11]-SRIF and des-amino acid1,2,5[D-Trp8,Iamp9],[^{125}I -Tyr11]-Cbm-SRIF bound at sst_1 receptors with median inhibitory concentration (IC_{50}) values of 17.1 ± 6 nM and 8.1 ± 1.3 nM, respectively. Furthermore, these analogues were shown to detect sst_1 -expressing tumours using *in vitro* receptor autoradiography [53]. These analogues were shown to be agonists since they inhibited forskolin-induced cAMP accumulation.

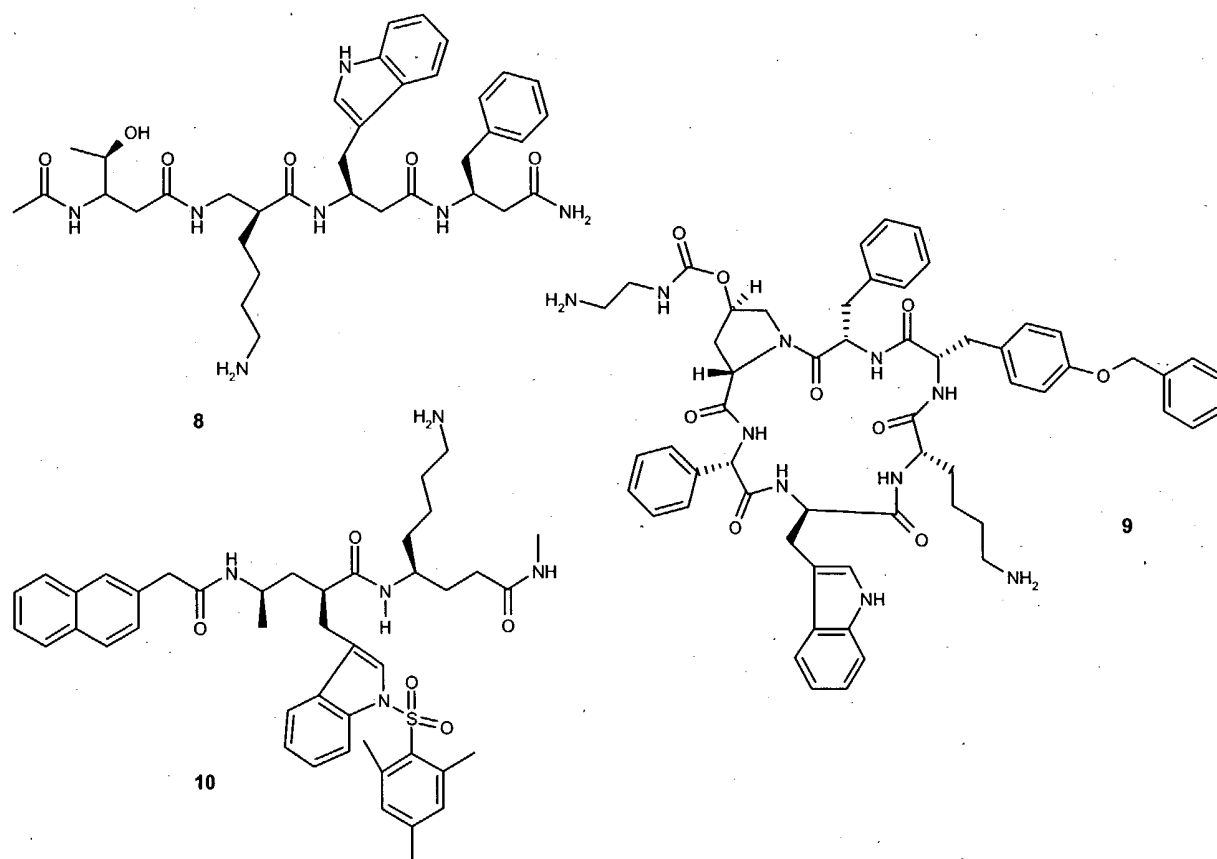
Rajeswaran *et al.* [54] carried out an *N*-methyl scan on the cyclic octapeptides, D-Phe5-c[Cys6-Phe7-D-Trp8-Lys9-Thr10-Cys11]Thr12-NH₂ (SRIF numbering, K_d 17.9 ± 2.5 nM, sst_3) and Tyr5-c[Cys6-Phe7-D-Trp8-Lys9-Thr10-Cys11]-Thr12-NH₂, in an attempt to block intramolecular hydrogen bonding sites and to increase the metabolic stability of these peptides. Displacement studies using cell membranes of Chinese hamster ovary (CHO)-K1 cells showed that *N*-methylation of Phe7, Thr10, Cys11 and Thr12 essentially eliminated binding affinity, whereas *N*-methylation of Tyr5 or D-Phe5 or Cys6 resulted in analogues with K_d values of < 20 nM at sst_3 receptors.

A long-acting cyclic SRIF analogue (c[GABA-Phe-Trp-D-Trp-Lys-Thr-Phe-GlyC3-NH₂], PTR-3173; compound 7) was recently described [55]. This novel peptide showed 1000- and 10,000-fold more potent *in vivo* inhibition of GH release compared with inhibition of glucagon or insulin secretion,

respectively. PTR-3173 bound with high affinity for sst_2 (3 ± 0.75 nM), sst_4 (7 ± 1.1 nM) and sst_5 (1 ± 0.05 nM). Furthermore, PTR-3173 is reported to be the first SRIF analogue that demonstrates selective *in vivo* inhibition between GH and insulin release. As an extension of this work, Gazal *et al.* [56] replaced the lactam bridge in PTR-3173 by a disulfide bond. This analogue exhibited excellent metabolic stability but a different receptor selectivity for sst_2 (28.7 ± 2.17 nM), sst_3 (15.6 ± 2.1 nM), sst_4 (> 1000 nM) and sst_5 (1.3 ± 0.08 nM).

Gademann *et al.* [57] designed and synthesised nonconstrained, linear β -peptides which incorporate the critical β -turn (Phe7-Trp8-Lys9-Thr10) of SRIF. The *N*-acetylamide (Ac β 3-HThr- β 3-HLys- β 3-HTrp- β 3-HPhe-NH₂; compound 8) exhibited a K_d at sst_4 of 83 nM and at least a 100-fold selectivity with respect to other ssts. Movement of the Lys moiety to the 3-position resulted in a decrease in sst_4 affinity by more than 1000-fold.

Unlike most previous studies to develop subtype-selective SRIF analogues, Reubi *et al.* [58] developed agonists that bind equally well to all SRIF receptor subtypes. The rationale being that such analogues could offer a therapeutic advantage in the treatment of multiple sst receptor-expressing tumours. The target compound of this investigation (H₂N-Tyr-c[D-Dab-Arg-Phe-Phe-D-Trp-Lys-Thr-Phe]; KE-108) exhibited high affinity for all SRIF receptor subtypes ($\text{sst}_1 = 2.6 \pm 0.4$ nM, $\text{sst}_2 = 0.9 \pm 0.1$ nM, $\text{sst}_3 = 1.5 \pm 0.2$ nM, $\text{sst}_4 = 1.6 \pm 0.1$ nM and $\text{sst}_5 = 0.65 \pm 0.1$ nM). The related 3-I-Tyr analogue of KE-108, KE-119, also demonstrated high affinity, albeit slightly lower affinity, at all ssts. These analogues act as agonists as shown by their ability to inhibit forskolin-stimulated cAMP production. The presence of the Tyr in position zero of the cyclic peptide offers significant utility in the development of radioactive analogues having the potential to identify tissues expressing sst_1 – sst_5 receptor subtypes. Researchers at Novartis [101] reported the development of a cyclic hexapeptide (compound 9) with a similar broad binding profile at ssts. This peptide analogue exhibited IC_{50} values of 9.3, 1, 1.5, > 100 and 0.16 nM at sst_1 – sst_5 , respectively. The compound was stated to inhibit GH and insulin-like growth factor (IGF)-1 release in



rats. Furthermore, this analogue inhibited tumour growth in a subcutaneous tumour model in nude mice.

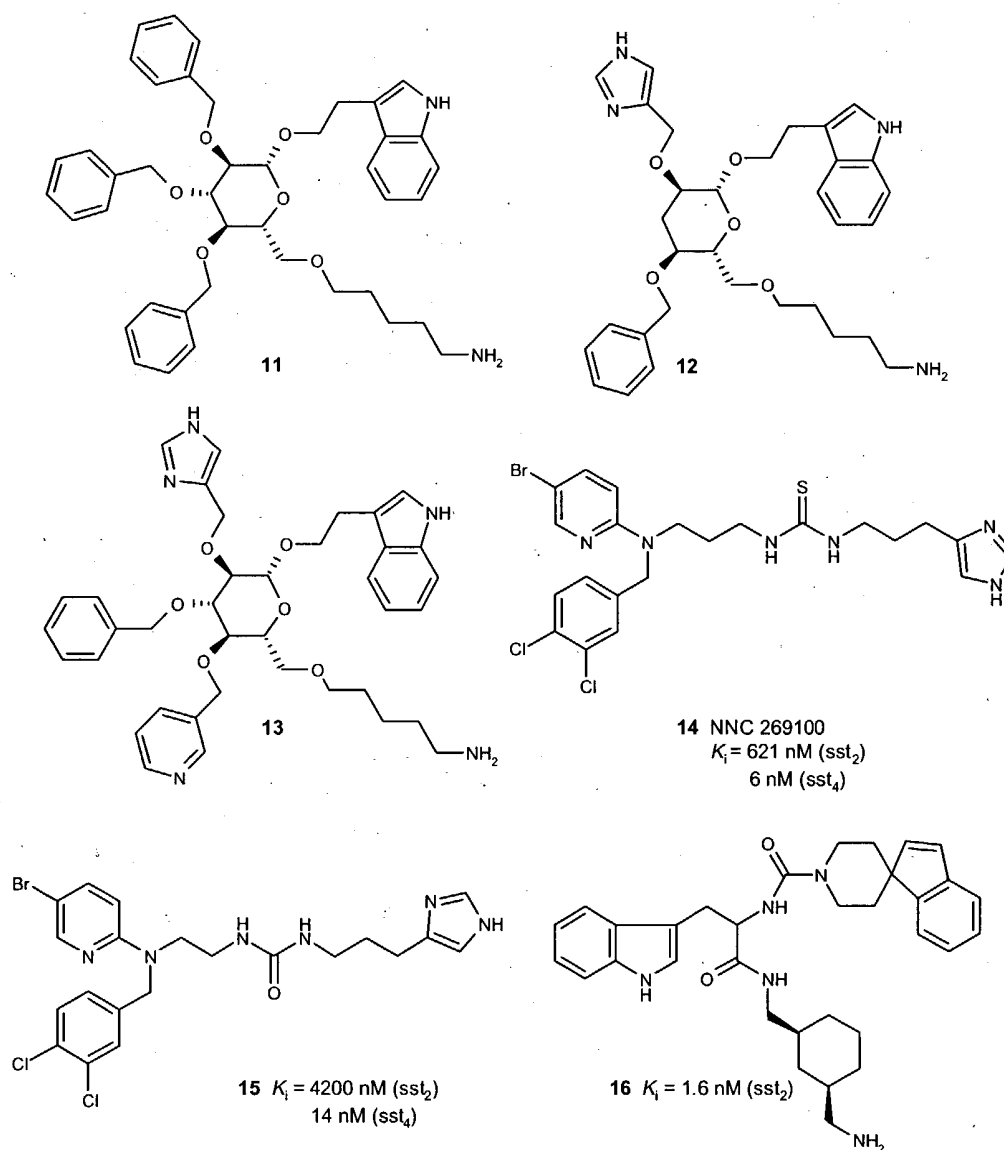
Seebach *et al.* [59] synthesised a series of simple γ -dipeptide derivatives for sst receptor binding affinity. These derivatives have the Trp side chain in the γ 2-position of the first amino acid and the Lys moiety at the γ 4-position of the second amino acid. The γ -dipeptide 10 showed moderate affinity for subtypes 1, 3 and 5 using a CCL-39 cell line. This work demonstrates that a simple γ -dipeptide can mimic SRIF, and these γ -dipeptides may be potentially useful in the development of metabolically stable peptidomimetics of SRIF.

A patent filed by researchers at Tulane [102] described the solid-phase synthesis of 18 cyclic peptides with affinity for ssts. The *N*-methylated analogue, [N-CH₃-D-Phe-D-Trp-Lys-Thr-Cys]-Thr-NH₂, exhibited K_d values of 316 nM for sst₁, 1.03 ± 0.26 nM for sst₂, 17.9 ± 2.5 nM for sst₃, > 1000 nM for sst₄, and 4.89 ± 1.4 nM for sst₅. This analogue was shown to act as an agonist in a rat pituitary cell assay with an IC₅₀ value of 0.32 nM in inhibition of GH release. These same researchers at Tulane [103] filed a patent on cyclic hydrophilic peptides for use in tumour detection and antitumour therapy. These analogues were rendered more hydrophilic by incorporating either a D-Lys-Lys or a D-Arg-Arg around a Tyr residue in the peptide backbone. One of the analogues (D-Lys-Tyr-Lys-Tyr-Lys-c[Cys-Phe-D-Trp-Lys-Thr-Cys]-Thr-NH₂; JF-0559) showed K_d values

of 0.3 nM for sst₁, 4.1 nM for sst₂, 6.4 nM for sst₃, 515 nM sst₄ and 13.4 nM for sst₅, respectively. This analogue exhibited an IC₅₀ value of 0.3 nM in a rat pituitary cell assay to assess agonist activity (sst₂-mediated) on GH release. These multiple Tyr-containing peptides are claimed to have a broader receptor-binding profile than previous derivatives. Also, the D-Tyr-containing analogues have high receptor affinity and the D-iodo-Tyr derivatives are more stable toward metabolic degradation than the corresponding L-analogues. Therefore, the D-iodo-Tyr derivatives retain the label for a longer period than the L-isomers.

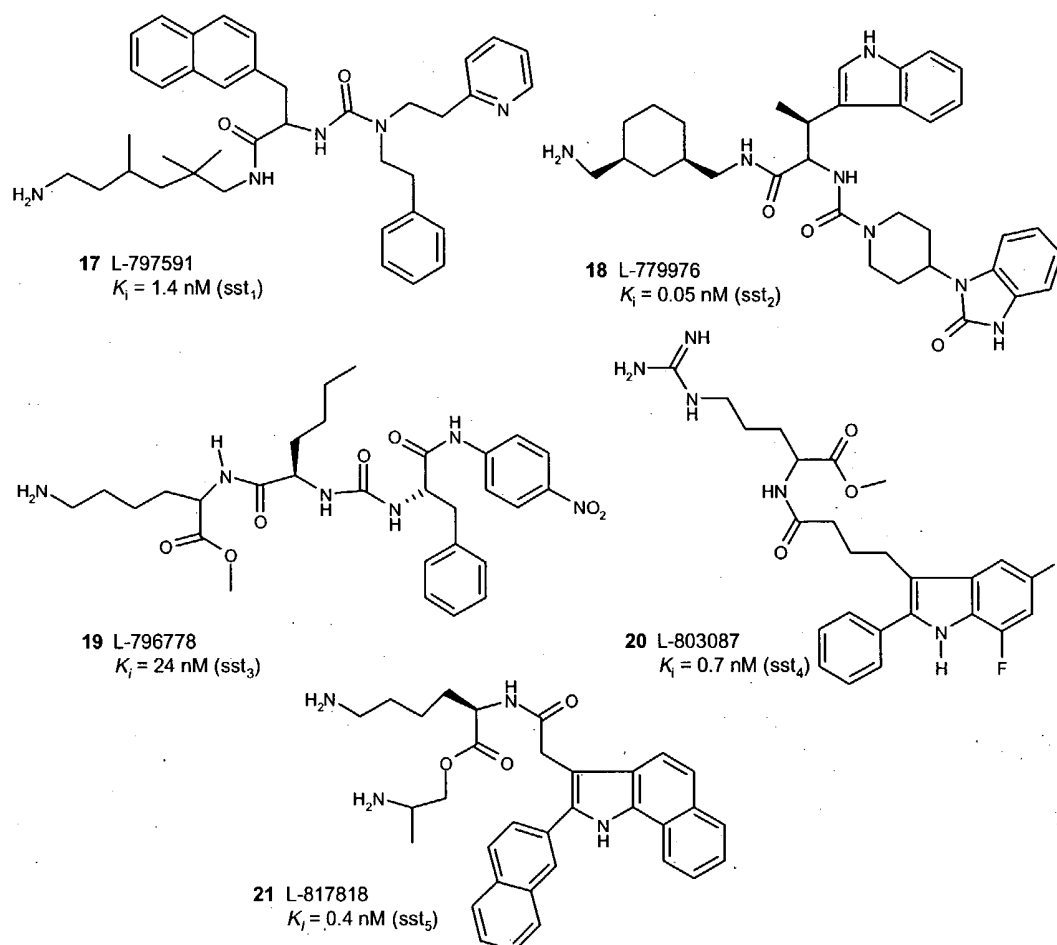
2.2 Peptide antagonists

The development of antagonists at ssts has lagged considerably behind the discovery of sst receptor agonists. The availability of selective antagonists is essential to fully characterise the functions of the individual SRIF receptor subtypes. Bass *et al.* [60] reported the first sst receptor antagonist in 1996. This cyclic octapeptide (AcNH-4-NO₂Phe5[D-Cys6-Tyr7-D-Trp8-Lys9-Thr10-Cys11]-D-Tyr12-NH₂; CYN-154806) inhibits the binding of SRIF at sst₂ and sst₅ receptors and antagonises SRIF agonist-stimulated inhibition of cAMP. Inversion of the chirality of disulfide-bridged octapeptide SRIF agonists at positions 5 and 6 (SRIF numbering: D5, L6 to L5, D6) led to the discovery of DC-3848, H-Nal-c[D-Cys-Pal-D-Trp-Lys-Val-Cys]-Nal-NH₂, a selective antagonist at sst₂ receptors [61]. Additional



studies from this group led to the cyclic octapeptide antagonist, H-Cpa-c[D-Cys6-Tyr7-D-Trp8-Lys9-Thr10-Cys11]-Nal-NH₂, an analogue with a dissociation constant for an inhibitor (K_i) of 26 nM at sst₂ receptors. This analogue showed potent antagonistic effects to SRIF in an *in vitro* rat pituitary assay [62]. Using a procedure similar to that described for the development of peptide agonists, Rajeswan *et al.* [63] carried out a systematic *N*-methylation approach of the lead antagonist, (H-Cpa-c[D-Cys6-Tyr7-D-Trp8-Lys9-Thr10-Cys11]-Nal-NH₂), to produce several antagonists with high affinity for sst₂, sst₃ and sst₅. The derivative containing an *N*-CH₃-Lys9 residue showed slightly lower binding affinity at sst₂, but this analogue was about fourfold more potent in an *in vitro* GH release assay. This analogue also inhibited sst₅ receptor calcium mobilisation in an *in vitro* assay in CHO-K1

cells. Replacement of the Lys9 residue by a 2,4-diaminobutyric acid group led to a compound with high selectivity at subtype 3 receptors. Further investigation of these SRIF antagonists led to sst₂ antagonists with high affinity for subtype 2 receptors. These same workers described related compounds in a 2002 patent [104]. The cyclic octapeptide Cpa-c[D-Cys-Tyr-D-Trp-*N*-CH₃Lys-Thr-Cys]-Nal exhibited K_i values of 1000 nM for sst₁, $5.51 \pm 1.85 \text{ nM}$ for sst₂, $115.1 \pm 16.9 \text{ nM}$ for sst₃, 1000 nM for sst₄, and $70.7 \pm 25.8 \text{ nM}$ for sst₅. Substitution of a hydrophilic His for the Nal12 at the C terminus resulted in a highly potent and selective sst₂ antagonist Cpa-c[D-Cys-Pal-D-Trp-*N*-CH₃Lys-Thr-Cys]-His-NH₂. An *in vitro* rat pituitary cell culture assay that measured inhibition of SRIF-inhibited GH release [64] was utilised to determine antagonistic activity of these compounds.



Reubi *et al.* [65] described the synthesis and evaluation of a family of disulfide-bridged octapeptide sst₃ receptor antagonists. The des-AA1,2,4,5,12,13-SRIF analogue, carbamoyl-desAA1,2,4,5,12,13[D-Cys3,Tyr7,D-Agl8 (CH₃, 2-naphthoyl)-SRIF, sst₃-ODN-8] exhibits comparable affinity to SRIF-28 at subtype 3 receptors. This analogue reversed SRIF-28-induced inhibition of forskolin-stimulated cAMP accumulation and antagonised SRIF-28-induced PLC stimulation. The Tyr7 residue was labelled with ¹²⁵I to yield [¹²⁵I-Tyr7]sst₃-ODN-8, which was shown to label sst₃-transfected CCL-39 cells.

In a recent report, researchers at Novartis evaluated the D-Tyr8 and L-Tyr8 isomers of CYN-154806 in recombinant human SRIF receptors and endogenous guinea-pig ileum receptors [66]. Radioligand-binding studies demonstrated nanomolar affinity at sst₂ receptors with 40- to 4500-fold greater affinity than at sst₁, sst₃ or sst₅ receptors. The L-isomer of CYN-154806 also demonstrated high affinity for subtype 5 receptors. Interestingly, both the D- and L-isomers demonstrated full agonist activity in inhibitory forskolin-induced cAMP accumulation. In the guinea-pig assay, the L-isomer showed agonist activity, whereas the D-isomer was inactive. This work implies that CYN-154806 is not a selective sst₂

antagonist and that the D- and L-Tyr8 isomers have high affinity for sst₅ receptors. Furthermore, CYN-154806 was shown to elicit agonist activity at sst₂.

2.3 Non-peptide agonists

The development of orally-active, non-peptide SRIF analogues with high selectivity for ssts has been an area of intense investigation in the last few years [11,67]. Hirschmann *et al.* [68] described the first non-peptide SRIF agonist in which a D-glucose scaffold was utilised to mimic the critical β -turn of SRIF. In compound 11, the key pharmacophoric groups occupy similar spatial orientations as found in octreotide; however, the compound demonstrated only weak agonist activity in AtT-20 cells. Additional studies from this group [69] demonstrated that compound 12 exhibited potent affinity for sst₄ receptors. Modification of compound 12 by the introduction of a 3-picolyl at C-4 and an (imidazol-4-yl)methyl group at C-2 led to compound 13. This analogue binds with high affinity and selectivity at sst₄ [70].

Ankersen *et al.* [71] reported the first non-peptide with high affinity for cloned human sst₄ receptors in 1998. The thiourea (14, NNC-269100) exhibited K_i values at sst₂ and sst₄ of 621 and 6 nM, respectively. Replacement of the

thiourea moiety in compound 14 by a urea function resulted in the sst_4 -selective analogue 15. This compound bound at sst_2 and sst_4 receptors with K_i values of 4200 and 14 nM, respectively. In this case, the urea moiety leads to retention of sst_4 affinity but with enhanced selectivity versus subtype 2 receptors. Compounds 14 and 15 showed full agonist activity and potently inhibited cAMP accumulation with median effective concentration (EC_{50}) values of 26 and 24 nM, respectively [72].

Yang *et al.* [73] reported the first non-peptide with high affinity and selectivity at human SRIF subtype 2 receptors. Compound 16 was a full agonist that potently inhibited forskolin-stimulated cAMP accumulation with an IC_{50} of 2 nM [74]. Additional work from the Merck group using combinatorial synthetic methods and high-throughput screening led to the discovery of non-peptides 17–21, which have high affinity and selectivity for all SRIF receptor subtypes [74,75]. The subtype 2-selective compound L-779976 potently inhibited forskolin-induced cAMP accumulation with an IC_{50} of 0.05 μM in CHO-K1 cells. This compound showed comparable potency to SRIF-14 in the inhibition of GH release from rat pituitary cells. Also, compound 18 inhibited arginine-stimulated glucagon release at a concentration of approximately 1000-fold less than blockade of insulin release. The sst_5 -selective compound L-817818 was effective in blocking insulin release in mouse pancreatic islets, but this compound was inactive in the inhibition of glucagon release. The β -Trp analogue (22, L-054522) displayed high affinity and selectivity at sst_2 receptors; however, a major limitation of this compound is its low oral bioavailability [76]. The Merck group postulated that elimination of the hydrogen bonding ability of the urea moiety through a two carbon tether would increase the bioavailability of analogues related in structure to L-054522. This approach led to the discovery of imidazolidin-2-one 23, a compound with an oral bioavailability of 64% [77].

Souers *et al.* [78] utilised a heterocyclic scaffold to mimic the β -turn of SRIF. Using this approach, all possible combinations of the Trp8 and Lys9 side chains of SRIF were attached to the scaffold to give a number of compounds with high affinity for ssts. Compound 24 exhibited an IC_{50} of 87 nM at sst_5 receptors.

Parallel synthesis was utilised to prepare a variety of imidazopyrazines and dihydroimidazopyrazines for SRIF receptor binding affinity in CHO-K1 cells [79]. The imidazopyrazine 25 exhibited moderate affinity (K_i of 360 ± 21 nM) at sst_5 , and this derivative was shown to be an agonist in the inhibition of forskolin-induced cAMP accumulation in CHO-K1 cells expressing sst_5 receptors. These same workers prepared additional 4-phenylimidazoles bearing a D-Trp *tert*-butyl ester group at the 2-position of the imidazole ring. Compound 26 was shown to have moderate affinity for subtype 3 receptors [80]. SAR studies on the sst_3 agonist 26 led to a series of related β -carboline analogues. Compound 27 was claimed to bind at ssts; however, no functional data were presented [105]. Related

4-phenylimidazoles, compounds 28 and 29, were described in two SCRAS patents [106,107]. The compounds were evaluated for sst_1 – sst_5 receptor binding affinity using [^{125}I Tyr11]-SRIF and agonist activity by inhibition of forskolin-induced cAMP accumulation; however, no specific data were given. Numerous other analogues related in structure to compounds 25 and 26 are reported in this SCRAS patent [107]. The tetrahydroimidazo[1,2-a]pyrazine 30 is a representative of the compounds described in this patent.

In a 2001 patent [108], scientists at Novartis described the synthesis of numerous hydantoins as SRIF receptor ligands. The trisubstituted-hydantoin 31 was claimed to be an sst_2 agonist. This analogue inhibited *in vitro* GH release in cultured pituitary cells and decreased serum GH and insulin levels in rats. In addition, compound 31 exhibited oral activity in models to predict anxiolytic, antidepressant, antipsychotic and anticonvulsant activity.

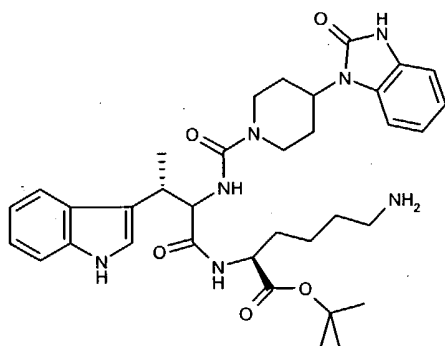
2.4 Non-peptide antagonists

The pseudosymmetry of the D-glucose moiety in compound 11 was proposed as the reason for the ability of this analogue to bind at several G-protein-coupled receptors [68]. The pseudosymmetry of the D-glucose group allows the sugar the freedom to adopt a variety of binding modes, therefore providing affinity for multiple G-protein-coupled receptors. Rohrer *et al.* [81] suggested that the D-glucose derivative 11 and related analogues bind with the precoupled form of ssts. This activated form is thought to be responsible for agonist activity in most receptor–ligand binding interactions at ssts. This hypothesis is supported by the fact that only a few SRIF antagonists are known.

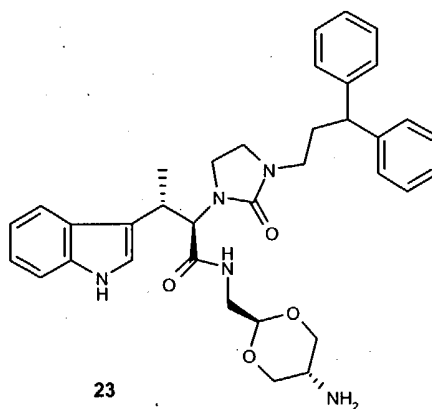
Researchers at Takeda Chemical Industries [109] described a series of tetrahydroquinolines as either SRIF agonists or antagonists. The phenylpiperazine 32 exhibited IC_{50} values of 9 and 0.8 nM, respectively, in displacement of [^{125}I Tyr11]-SRIF in CHO-K1 cells at sst_2 and sst_3 , however, no functional data were presented to classify this compound as an agonist or an antagonist. A subsequent patent from Takeda [110] described related derivatives. The *bis*-indolyltetrahydroquinoline 33 demonstrated IC_{50} values of 0.3, 80 and 400 nM at sst_2 , sst_3 and sst_4 , respectively.

Patents filed by workers at SCRAS [111,112] described the synthesis of 2-arylimino-2,3-dihydrothiazole derivatives as ligands for ssts. Thiazoles 34 and 35 were reported to have K_i values of < 200 nM in displacement of [^{125}I Tyr11]-SRIF-14 in CHO-K1 cells expressing ssts.

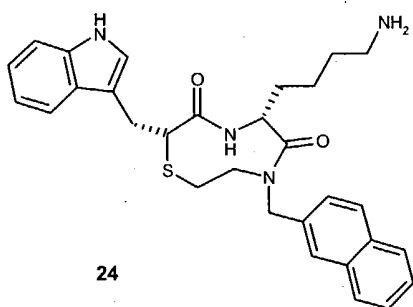
β -Carbolines, related in structure to compound 27, were reported in a Novartis patent [113]. These derivatives exhibit high binding affinity for sst_3 receptors and function as antagonists. The 4-phenylimidazo- β -carboline 36 was shown to have pK_d ($-\log_{10}$ dissociation constant) values of 8.69 and 8.30 nM for human and mouse subtype 3 receptors, respectively. This compound demonstrated over 400-fold greater selectivity at sst_3 receptors compared with other SRIF receptor subtypes, and compound 36 and



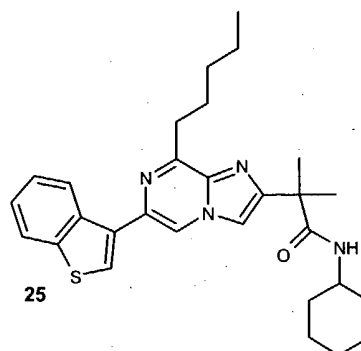
22 L-054522



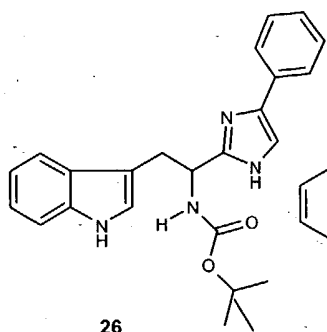
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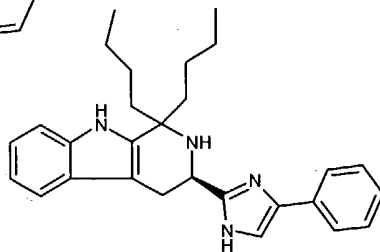
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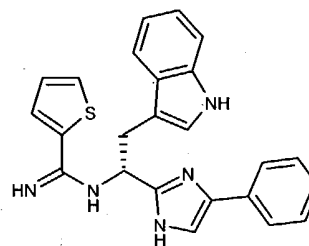
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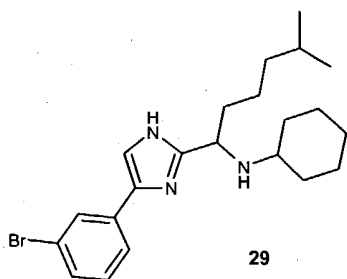
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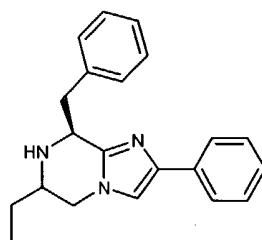
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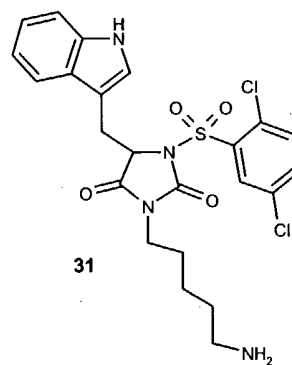
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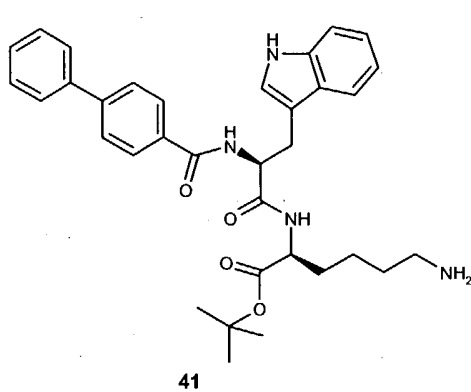
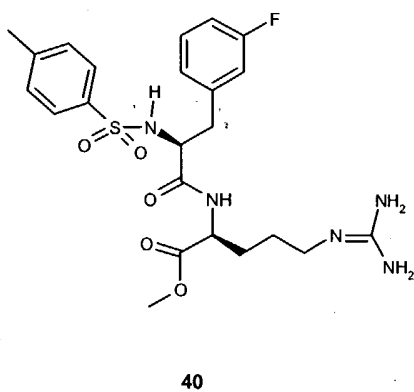
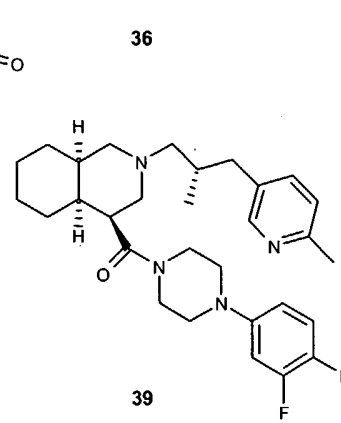
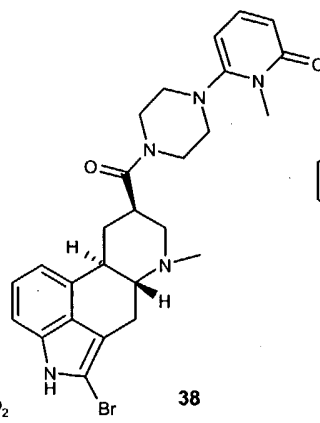
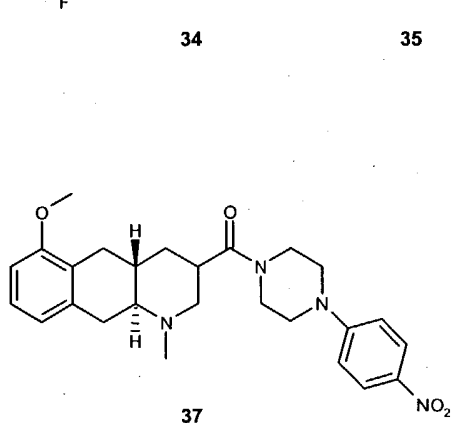
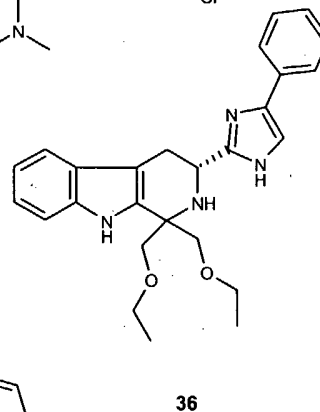
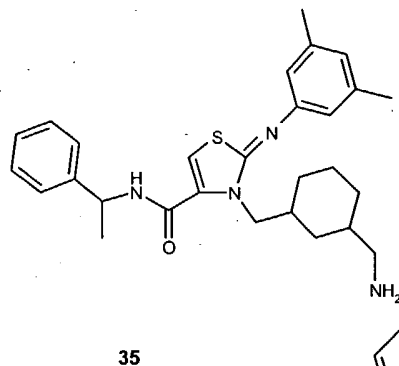
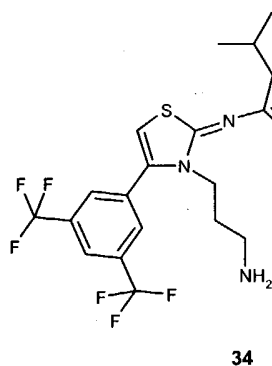
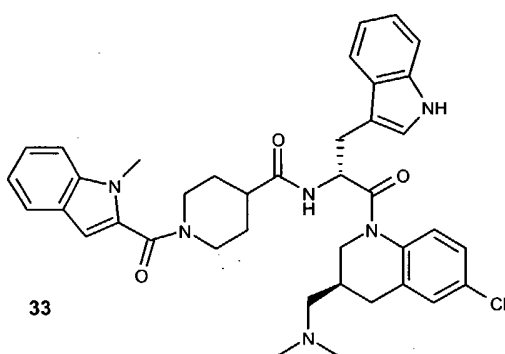
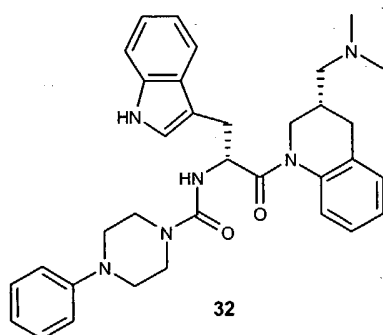


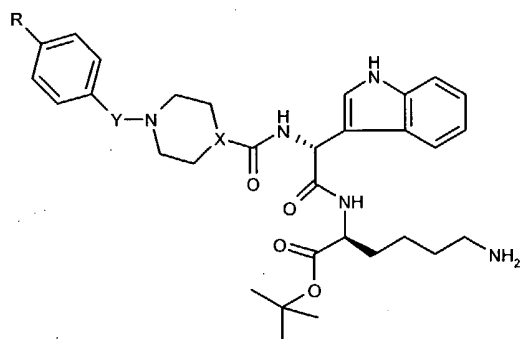
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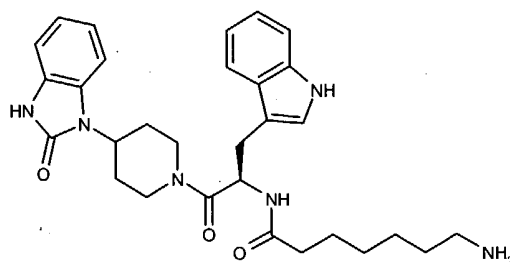
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Somatostatin receptor agonists and antagonists

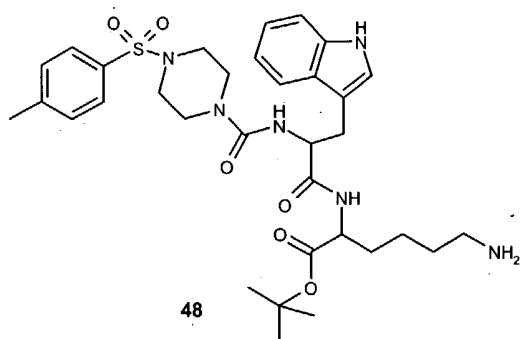




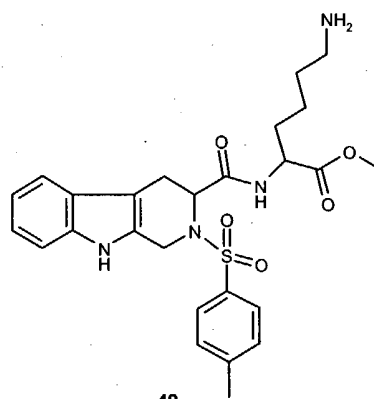
- 42 X = N, Y = SO₂, R = H; IC₅₀ = 2.9 nM
 43 X = N, Y = SO₂, R = CH₃; IC₅₀ = 9.2 nM
 44 X = N, Y = CO, R = H; IC₅₀ = 6.9 nM
 45 X = N, Y = CO, R = CH₃; IC₅₀ = 4.4 nM
 46 X = CH₃, Y = SO₂, R = H; IC₅₀ = 3.2 nM



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48



49

related analogues were evaluated for CNS activity in a variety of animal models.

A Novartis patent [114] described the synthesis of octahydrobenzo[g]quinolines as selective sst₁ antagonists. The 4-phenylpiperazino-octahydrobenzo[g]quinoline 37 exhibited high affinity for native rat subtype 1 receptors (pIC₅₀ = 9.1) with little affinity for other receptor subtypes. This compound was active in animal models that predict anxiolytic, antipsychotic and affective disorders such as mania. A Novartis patent [115] claimed that piperazineamides were sst₁ antagonists. The ergoline 38 exhibited a pIC₅₀ value of 9.7 at native rat subtype 1 receptors and pIC₅₀ values of 9 and 8.8 at native and recombinant human sst₁ receptors. This compound lowered aggressive behaviour in the matched pairs situation test and reversed social withdrawal in the intruder mouse test. Activity in these tests indicated therapeutic potential for anxiety, psychosis and mania. Decahydroisoquinolines that incorporate the 4-phenylpiperazincarbonyl moiety at the 4-position were described in another 2001 Novartis patent [116]. Compound 39 was described as an sst₃ antagonist with activity in several tests that predict CNS activity.

Using the initial lead molecule 40, researchers at Pfizer developed the D-Trp analogue 41 as the first non-peptide sst₂ antagonist [82]. These workers found that small structural changes in these molecules can convert antagonists into agonists. On the

basis of this work, the Pfizer group modified the Merck sst₂ agonist L-054522 (22) in an attempt to find related sst₂ antagonists. These workers used the structural features found in L-054522 to retain potency but incorporated the structural components of 40 to impart antagonist activity. The result of this approach led to the full sst₂ antagonists 42 – 46. Antagonist activity of these compounds was determined in pituitary cells in which cAMP content was measured. The synthesis of other close analogues of L-054522 was described in a Pfizer patent application in 2002 [117]. Representative compounds include the benzimidazol-2-one 47, which is presumably an sst₂ antagonist. These compounds apparently increase the secretion of GH in small pigs. Other related analogues (48 and 49) were also described in Pfizer 2002 patent applications [118–119]. These analogues are reportedly sst₂ antagonists.

A re-evaluation of the radioligand binding and functional characteristics of the Merck agonist L-054522 and the Pfizer antagonists 42 and 46 was performed by researchers at Novartis [83]. In this study, all three compounds demonstrated agonist activity in the inhibition of forskolin-induced cAMP accumulation and the increase in luciferase reporter gene expression in CHO cells. The results of the binding studies revealed that L-054522 was 10- to 100-fold more potent than 42 and 46 in the displacement of radioligands from rat cortex

and human ss_{2} receptors, which were expressed in CCL-39 cells. Furthermore, these compounds were also shown to bind with high affinity at ss_{5} .

3. Expert opinion

SRIF is widely distributed in the CNS, the periphery and in various tumour cells. The two biologically active forms, SRIF-14 and SRIF-28, elicit their effects through five subtypes (ss_{1} – ss_{5}) of G-protein-coupled receptors. However, most cells contain more than one receptor subtype. Furthermore, homodimerisation and heterodimerisation of SRIF receptors may alter receptor function. The closely related peptide CST binds equally well to SRIF receptor subtypes. Whether specific CST receptors exist remains to be determined. Peptide analogues have been discovered by peptide backbone modification and truncation; however, the problems of enzymatic liability, and suitable delivery forms of these analogues present major challenges. The work of Merck scientists that led to the development of SRIF subtype-specific non-peptide agonists was a significant achievement. Furthermore, the discovery of non-peptide antagonists by researchers at Pfizer will facilitate

the study of the role and function of individual SRIF receptor subtypes. However, additional research is necessary to fully characterise these novel antagonists. Peptide analogues such as octreotide have demonstrated clinical effectiveness in the treatment of acromegaly and various tumours. Other potential therapeutic uses of SRIF analogues include treatment of disorders of the CNS, modulation of glucose homeostasis, regulation of insulin and glucagon secretion, tumour diagnosis and treatment and diabetic retinopathy.

In a very short time, subtype-selective ligands have been discovered for all SRIF receptor subtypes. Many of these compounds offer new options in the treatment of various disease states. In addition, these compounds provide valuable tools to study the role and function of SRIF and its receptors throughout the body. Research on SRIF should continue to be a fertile area of study for the future.

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Bibliography

Papers of special note have been highlighted as either of interest (*) or of considerable interest (**) to readers.

- BRAZEAU P, VALE W, BURGUS R *et al.*: Hypothalamic polypeptide that inhibits the secretion of immunoreactive pituitary growth hormone. *Science* (1973) 179:77-79.
- DE LECEA L, CRIADO JR, PROSPERO-GARCIA O *et al.*: A cortical neuropeptide with neuropeptide with neuronal depressant and sleep-modulating properties. *Nature* (1996) 381:242-245.
- SPIER AD, DE LECEA L: A member of the somatostatin neuropeptide family with distinct physiological functions. *Brain Res. Rev.* (2000) 33(2,3):228-241.
- FUKUSUMI S, KITADA C, TAKEKA S *et al.*: Identification and characterization of a novel human cortistatin-like peptide. *Biochem. Biophys. Res. Commun.* (1997) 232:157-163.
- SIEHLER S, SEUWEN K, HOYER D: [125 I]-Tyr 10 -cortistatin labels all five somatostatin receptors. *Naunyn-Schmiedeberg's Arch. Pharmacol.* (1998) 375(5):483-489.
- PUEBLA L, MOUCHANTAF R, SASI R *et al.*: Processing of rat preprocortistatin in mouse AtT-20 cells. *J. Neurochem.* (1999) 73:1273-1277.
- DEGHENGI R, PAPOTTI M, GHIGO E, MUCCIOLI G: Cortistatin, but not somatostatin, binds to growth hormone secretagogue (GSH) receptors of human pituitary. *J. Endocrinol. Invest.* (2001) 24:RC1-RC3.
- DALM VA, VAN HAGEN MP, VAN KOETSVELD PM *et al.*: Cortistatin rather than somatostatin as a potential ligand for somatostatin receptors in the human immune system. *J. Clin. Endocrinol. Metabol.* (2003) 88:270-276.
- * Data presented indicating that CST may be the endogenous ligand at ss_{2} in the human immune system.
- HOYER D, BELL GI, BERELOWITZ M *et al.*: Classification and nomenclature of somatostatin receptors. *Trends Pharmacol. Sci.* (1995) 16:86-88.
- BELL GI, REISINE T: Molecular biology of somatostatin receptors. *Trends Neurosci.* (1993) 16:34-38.
- HANNON JP, NUNN C, STOTZ B *et al.*: Drug design at peptide receptors. *J. Mol. Neurosci.* (2002) 18:15-27.
- * A review of SRIF and SRIF receptor ligands.
- PIWKO C, THOSS VS, PROBST A, HOYER D: Localization and pharmacological characterization of somatostatin recognition sites in the human cerebellum. *Neuropharmacology* (1996) 35:713-723.
- SCHINDLER M, KIDD EJ, CARRUTHERS AM *et al.*: Molecular cloning and functional characterization of a rat somatostatin ss_{2B} receptor splice variant. *Br. J. Pharmacol.* (1998) 125:209-217.
- SARRET P, GOTTO JM, VINCENT JP, MAZELLA J, BEAUDET A: Preferential expression of ss_{2A} over ss_{2B} somatostatin receptor splice variant in rat brain and pituitary. *Neuroendocrinology* (1998) 68:37-43.
- PATEL YC: Molecular pharmacology of somatostatin receptor subtypes. *J. Endocrinol. Invest.* (1997) 20:348-367.
- PATEL YC, GREENWOOD MT, PANETTA R, DEMCHYSHYN L, NIZNIK H, SRIKANT CB: The somatostatin receptor family: a mini review. *Life Sci.* (1995) 57:1249-1265.
- PATEL YC: Somatostatin and its receptor family. *Frontiers Neuroendocrinol.* (1999) 20:157-158.
- MURTHY KS, COY DH, MAKHOULF G: Somatostatin receptor mediated signaling in smooth muscle. *J. Biol. Chem.* (1996) 271:23458-23463.
- SCHWEITZER R, MADAMBE S, SIGGINS GR: Arachidonic acid metabolites as mediators of somatostatin-induced increase of neuronal m-current. *Nature* (1990) 346:464-467.

20. PFEIFFER M, KOCH T, SCHODER H *et al.*: Homo- and heterodimerization of somatostatin receptor subtypes. Inactivation of sst₃ receptor function by heterodimerization with sst_{2A}. *J. Biol. Chem.* (2001) 276:14027-14036.
21. PFEIFFER M, KOCH T, SCHRODER H, LAUGSCH M, HOLLT V, SCHULZ S: Heterodimerization of somatostatin and opioid receptors cross-modulates phosphorylation, internalization, and desensitization. *J. Biol. Chem.* (2002) 277:19762-19772.
22. BREDER CD, YAMADA YY, YASUDA K *et al.*: Differential expression of somatostatin receptor subtypes in brain. *J. Neurosci.* (1992) 12:3920-3924.
23. KONG H, DE PAOLI AM, BREDER CD, YASUDA K, BELL GI, REISINE T: Differential expression of messenger RNA's for somatostatin receptor subtypes SSTR1, SSTR2 and SSTR3 in adult rat brains: analysis by RNA blotting and *in situ* hybridization histochemistry. *Neuroscience* (1994) 59:175-184.
24. THOSS VS, PEREZ J, DUC D, HOYER D: Embryonic and postnatal mRNA distribution of five receptor subtypes in rat brain. *Neuropharmacology* (1995) 34:1673-1688.
25. DAY R, DONG W, PANETTA R, KRAICER J, GREENWOOD MT, PATEL YC: Expression of mRNA for somatostatin receptor (sstr) Types 2 and 5 in individual rat pituitary cells. A double labeling *in situ* hybridization analysis. *Endocrinology* (1995) 136:5232-5235.
26. HOYER D, LUBBERT H, BRUNS C: Molecular pharmacology of somatostatin receptors. *Naunyn Schmiedebergs Arch. Pharmacol.* (1994) 350:441-453.
27. LE ROMANCER M, CHERIFI Y, LAVASSEUR S *et al.*: Messenger RNA expression of somatostatin receptor subtypes in human and gastric mucosae. *Life Sci.* (1996) 58:1091-1098.
28. BRUNO JF, XU Y, SONG J, BERELOWITZ M: Tissue distribution of somatostatin receptor subtype messenger ribonucleic acid in rat. *Endocrinology* (1993) 133:2561-2567.
29. CURTIS SB, HEWITT J, YAKUBOVITZ S, ANZARUT A, YSIANG YN, BUCHAN AM: Somatostatin receptor subtype expression and function in human vascular tissue. *Am. J. Physiol.* (2000) 278:1815-1822.
30. BATES CM, KEGG H, PETREVSKEI C, GRADY S: Expression of somatostatin receptors 3, 4, and 5 in mouse kidney proximal tubules. *Kidney Int.* (2003) 63:53-63.
31. LIU S, CRIDER AM, TANG C, HO B, ANKERSEN M, STIDSEN CE: 2-Pyridylthioureas: novel non-peptide somatostatin agonists with sst₄ selectivity. *Curr. Med. Chem.* (1999) 6:401-410.
32. CULLER MD, TAYLOR JE, MOREAU JP: Somatostatin receptor subtypes: targeting functional and therapeutic specificity. *Ann. Endocrinol.* (2002) 63(2Part3):2S5-2S12.
33. STROWSKI MZ, PARMAR RM, BLAKE AD, SCHAEFFER JM: Somatostatin inhibits insulin and glucagon secretion via two receptor subtypes: an *in vitro* study of pancreatic islets from somatostatin receptor 2 knockout mice. *Endocrinology* (2000) 141:111-117.
- Data presented showing that insulin secretion was mediated via sst₅, whereas glucagon release was regulated by sst₂.
34. STROWSKI MZ, KOHLER M, CHEN HY *et al.*: Somatostatin receptor subtype 5 regulates insulin secretion and glucose homeostasis. *Mol. Endocrinol.* (2003) 17:93-106.
35. STROWSKI MZ, DASHKEVICZ MP, PARMAR RM *et al.*: Somatostatin receptor subtypes 2 and 5 inhibit corticotropin-releasing hormone-stimulated adrenocorticotropin secretion from AtT-20 cells. *Neuroendocrinology* (2002) 75:339-346.
36. DRUCKER DJ: Biological actions and therapeutic potential of the glucagon-like peptides. *Gastroenterology* (2002) 122:531-544.
37. STARK A, ROLF M: Somatostatin inhibits glucagon-like peptide-1 induced insulin secretion and proliferation of RINm5F insulinoma cells. *Regul. Pept.* (2002) 108:97-102.
38. CHISHOLM C, GREENBERG GR: Somatostatin-28 regulates GLP-1 secretion via somatostatin receptor subtype 5 in rat intestinal cultures. *Am. J. Physiol.* (2002) 283(2Part1):E311-E317.
39. DI VITO L, BROGLIO F, BENSO A *et al.*: The GH-releasing effect of ghrelin, a natural GH secretagogue, is only blunted by the infusion of exogenous somatostatin in humans. *Clin. Endocrinol.* (2002) 56:643-648.
40. BROGLIO F, VAN KOELSVELD P, BENSON A *et al.*: Ghrelin secretion is inhibited by either somatostatin or cortistatin in humans. *J. Clin. Endocrinol. Metabol.* (2002) 87:4829-4832.
41. VASILAK A, GEORGOUSI Z, THERMOS K: Somatostatin receptors (sst₂) are coupled to G_o and modulate GTPase activity in rabbit retina. *J. Neurochem.* (2003) 84:625-632.
42. DAL MONTE M, PETRUCCI C, COZZI A, ALLEN JP, BAGNOLI P: Somatostatin inhibits potassium-evoked release by activation of sst₂ somatostatin receptor in mouse retina. *Naunyn Schmiedebergs Arch. Pharmacol.* (2003) 367:186-192.
43. VASILAKI A, MOURATIDOU M, SCHULZ S, THERMOS K: Somatostatin mediates nitric oxide production by activating sst₂ receptors in rat retina. *Neuropharmacology* (2002) 43:899-909.
44. REUBI JC, WASER B, SCHAEER JC, LAISSUE JA: Somatostatin receptor sst₁ - sst₅ expression in normal and neoplastic human tissues using receptor autoradiography with subtype-selective ligands. *Eur. J. Nucl. Med.* (2001) 28:836-846.
45. TEIJEIRO R, RIOS R, COSTOYA J *et al.*: Activation of human somatostatin receptor 2 promotes apoptosis through a mechanism that is independent from induction of p53. *Cell Physiol. Biochem.* (2002) 12:31-38.
46. HALL GH, TURNBULL LW, RICHMOND I, HELBOE L, ATKIN SL: Localization of somatostatin and somatostatin receptors in benign and malignant ovarian tumors. *Br. J. Cancer* (2002) 87:86-90.
47. HANSSON J, BJARTELL A, GADALEANU V, DIZEYI N, ABRAHAMSSON PA: Expression of somatostatin receptor subtype 2 and 4 in human benign prostatic hyperplasia and prostatic cancer. *Prostate* (2002) 53:50-59.
48. LUTHERMAN K, HACKSELL U: Chapter 14. Peptides and peptidomimetics. In: *A Textbook of Drug Design and Development*. Krogsgaard-Larsen P, Liljeferos T, Madsen U (Eds), Harwood Academic Publishers, Amsterdam (1996):386-406.
49. VALE W, RIVIER J, LING N, BROWN M: Biologic and immunologic activities and applications of somatostatin analogues. *Metabolism* (1978) 27:1392-1401.

50. VEBER DF, SAPERSTEIN R, NUTT RF *et al.*: Super active hexapeptide analogues of somatostatin. *Life Sci.* (1984) 34:1371-1378.
51. BAUER W, BRINER U, DOEPFNER W *et al.*: A very potent and selective octapeptide analogue of somatostatin with prolonged action. *Life Sci.* (1982) 31:1133-1140.
52. LIAPAKIS G, HOEGER C, RIVIER J, REISINE T: Development of a selective agonist at the somatostatin receptor subtype sst₁. *J. Pharmacol. Exp. Ther.* (1996) 276:1089-1094.
53. RIVIER JE, HOEGER C, ERCHEGYI J *et al.*: Potent undecapeptide agonists selective for somatostatin receptor 1 (sst₁). *J. Med. Chem.* (2001) 44:2238-2246.
- Report on des-amino acid (1,2,5)-SRIF analogues having high affinity for sst₁.
54. RAJESWARAN WG, HOCART SJ, MURPHY WA, TAYLOR JE, COY DH: *N*-Methyl scan of somatostatin octapeptide agonists produces interesting effects on receptor subtype specificity. *J. Med. Chem.* (2001) 44:1416-1421.
55. AFARGAN M, JANSON ET, GELERMAN G *et al.*: Novel long-acting somatostatin analogues with endocrine selectivity: potent suppression of growth hormone but not insulin. *Endocrinology* (2001) 142(1):477-486.
- Data presented to show that PTR-3173 selectively inhibited GH release compared to insulin or glucagon release.
56. GAZAL S, GELERMAN G, ZIV O *et al.*: Human somatostatin receptor specificity of backbone-cyclic analogues containing novel sulfur building units. *J. Med. Chem.* (2002) 45:1665-1671.
57. GADEMANN K, KIMMERLIN T, HOYER D, SEEBACH D: Peptide folding induces high and selective affinity of a linear and small β -peptide to the human somatostatin receptor 4. *J. Med. Chem.* (2001) 44:2460-2468.
58. REUBI JC, EISENWIENER KP, RINK H, WASER B, MACKE HR: A new peptidic somatostatin agonist with high affinity to all five somatostatin receptors. *Eur. J. Pharmacol.* (2002) 456:45-49.
59. SEEBACH D, SCHAEFFER L, BRENNER M, HOYER D: Design and synthesis of γ -dipeptide derivatives with submicromolar affinities for human somatostatin receptors. *Angew. Chem. Int. Ed.* (2003) 42:776-778.
- Report on γ -dipeptides as metabolically stable peptidomimetics of SRIF.
60. BASS RT, BUCKWALTER BL, PATEL BP *et al.*: Identification and characterization of novel somatostatin antagonists. *Mol. Pharmacol.* (1996) 50:709-715.
61. HOCART SJ, JAIN R, MURPHY WA, TAYLOR JE, MORGAN B, COY DH: Potent antagonists of somatostatin: synthesis and biology. *J. Med. Chem.* (1998) 41:1146-1154.
62. HOCART SJ, JAIN R, MURPHY WA, TAYLOR JE, COY DH: Highly potent cyclic disulfide antagonists of somatostatin. *J. Med. Chem.* (1999) 42:1863-1871.
63. RAJESWAN WG, HOCART SJ, MURPHY WA, TAYLOR JE, COY DH: Highly potent and subtype selective ligands derived by *N*-methyl scan of a somatostatin antagonist. *J. Med. Chem.* (2001) 44:1305-1311.
- Systematic *N*-methyl scan of SRIF analogues to obtain sst₂, sst₃, and sst₅ antagonists.
64. RAJESWAN WG, MURPHY WA, TAYLOR JE, COY DH: Exploration of the D-Trp-*N*-MeLys motif in the search for potent somatostatin antagonists. *Bioorg. Med. Chem.* (2002) 10:2023-2030.
65. REUBI JC, SCHAEFER JC, WENGER S *et al.*: sst₃-Selective potent peptidic somatostatin receptor antagonists. *Proc. Natl. Acad. Sci. USA* (2000) 97:13973-13978.
66. NUNN C, SCHOEFFTER P, LANGENEGGER D, HOYER D: Functional characterization of the putative somatostatin sst₂ receptor antagonist CYN-154806. *Naunyn Schmiedeberg's Arch. Pharmacol.* (2003) 367:1-9.
- Re-evaluation of the putative sst₂ antagonist CYN-154806.
67. CRIDER AM: Recent advances in the development of non-peptide somatostatin receptor ligands. *Mini Rev. Med. Chem.* (2002) 2:507-517.
- Review on SRIF and SRIF receptor ligands.
68. HIRSCHMANN R, NICOLAOU K, PIETRANICO S *et al.*: Non-peptidic peptidomimetics with β -D-glucose scaffolding. A partial somatostatin agonist bearing a close structural relationship to a potent, selective substance P antagonist. *J. Am. Chem. Soc.* (1992) 114:9217-9218.
69. HIRSCHMANN R, HYNES J Jr, CICHY-KNIGHT MA *et al.*: Modulation of receptor and receptor subtype affinities using diastereomeric and enantiomeric monosaccharide scaffolds as a means to structural and biological diversity. A new route to ether synthesis. *J. Med. Chem.* (1998) 41:1382-1391.
70. PRASAD V, BIRZIN ET, MC VAUGH CT *et al.*: Effects of heterocyclic aromatic substituents on binding affinities at two sites of somatostatin (SRIF) receptors. Correlation with electrostatic potential of the substituents. *J. Med. Chem.* (2003) 46:1858-1869.
- D-Glucose-derived non-peptides with high affinity for sst₄.
71. ANKERSEN M, CRIDER M, LIU S, HO B, ANDERSEN HS, STIDSEN C: Discovery of a novel non-peptide somatostatin agonist with SST₄ selectivity. *J. Am. Chem. Soc.* (1998) 120:1368-1373.
72. LIU S, TANG C, HO B, ANKERSEN M, STIDSEN CE, CRIDER AM: Non-peptide somatostatin agonists with sst₄ selectivity: synthesis and structure-activity relationships of thioureas. *J. Med. Chem.* (1998) 41:4693-4705.
73. YANG L, GUO L, PASTERNAK A *et al.*: Spiro[1*H*-indene-1,4'-piperidine]derivatives as potent and selective non-peptide human somatostatin receptor subtype 2 (sst₂) agonists. *J. Med. Chem.* (1998) 41:2175-2179.
74. ROHRER SP, BIRZIN ET, MOSLEY RT *et al.*: Rapid identification of subtype-selective agonists of the somatostatin receptor through combinatorial chemistry. *Science* (1998) 282:737-740.
- This paper describes the discovery of SRIF non-peptide analogues at all ssts.
75. BERK SC, ROHRER SP, DEGRADO SJ *et al.*: A combinatorial approach toward the discovery of non-peptide subtype-selective somatostatin receptor ligands. *J. Comb. Chem.* (1999) 1:388-396.
76. YANG L, BERK SC, ROHRER SP *et al.*: Synthesis and biological evaluation of potent peptidomimetics selective for somatostatin receptor subtype 2. *Proc. Natl. Acad. Sci. USA* (1998) 95:10836-10841.
77. PASTERNAK A, PAN Y, MARINO D *et al.*: Potent, orally-bioavailable somatostatin agonists: good absorption achieved by urea backbone cyclization. *Bioorg. Med. Chem. Lett.* (1999) 9:491-496.
78. SOUERS AJ, VIRGILLO AA, ROSENQUIST A *et al.*: Identification of a potent heterocyclic ligand to somatostatin receptor subtype 5 by synthesis and screening of β -turn mimetic libraries. *J. Am. Chem. Soc.* (1999) 121:1817-1825.
79. GARCERA-CONTOUR MO, POITOUT L, MOINET C *et al.*: Synthesis of imidazopyrazines as ligands for the human somatostatin receptor subtype 5. *Bioorg. Med. Chem. Lett.* (2001) 11:741-745.

80. MOINET C, CONTOUR-GALCERA MO, POITOUT L *et al.*: Novel non-peptide ligands for the somatostatin sst₃ receptor. *Bioorg. Med. Chem. Lett.* (2001) 11:991-996.
81. LIU J, UNDERWOOD DJ, CASCIERI MA *et al.*: Synthesis of a substance P antagonist with a somatostatin scaffold: factors affecting agonism/antagonism at GPCRs and the role pseudosymmetry. *J. Med. Chem.* (2000) 43:3827-3831.
82. HAY BA, COLE BM, DICAPUA F *et al.*: Small molecule somatostatin receptor subtype 2 antagonists. *Bioorg. Med. Chem. Lett.* (2001) 11:2731-2734.
- This paper reports the first non-peptide sst₂ antagonist.
83. NUNN C, LANGENEGGER D, HURTH K, SCHMIDT K, FEHLMANN D, HOYER D: Agonist properties of putative small-molecule somatostatin sst₂ receptor-selective antagonists. *Eur. J. Pharmacol.* (2003) 465:211-218.
- Re-evaluation of putative sst₂ antagonists. These analogues were shown to have sst₂ agonist activity and affinity for sst₅ receptors.

Patents

Patents of special note have been highlighted as being of interest (•) to readers.

101. NOVARTIS AG: WO0210192 (2002).
102. TULANE EDUCATIONAL FUND: WO0281499 (2002).
103. TULANE EDUCATIONAL FUND: US6465613 (2002).
- Hydrophilic peptide analogues of SRIF for tumor detection and antitumor therapy.
104. TULANE EDUCATIONAL FUND: WO0272602 (2002).
105. SCRAS: WO9964420 (1999).
106. SCRAS: WO994401 (1999).
107. SCRAS: WO0210140 (2002).
108. NOVARTIS AG: WO0185718 (2001).
- Use of a hydantoin scaffold for the development of non-peptide SRIF agonists.
109. TAKEDA CHEM. IND. LTD: JP12226373 (2000).
110. TAKEDA CHEM. IND. LTD: WO0125228 (2001).

111. SCRAS: WO0107424 (2001).
112. SCRAS: WO02055510 (2002).
113. NOVARTIS AG: WO02081471 (2002).
- β -Carboline antagonists with high affinity and selectivity for sst₃.
114. NOVARTIS AG: US5885988 (1999).
- Octahydrobenzo[*g*]quinoline antagonists with high affinity and selectivity for sst₁.
115. NOVARTIS AG: US6221870 (2001).
116. NOVARTIS AG: WO0170731 (2001).
117. PFIZER: US0091090 (2002).
118. PFIZER: US0016298 (2002).
119. PFIZER: US0091125 (2002).

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